THE LANCET

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: He Z, Ren L, Yang J, et al. Seroprevalence and humoral immune durability of anti-SARS-CoV-2 antibodies in Wuhan, China: a longitudinal, population-level, cross-sectional study. *Lancet* 2021; **397:** 1075–84.

Supplementary materials

Supplementary Methods

Cumulative infection rate calculation

The seroprevalence of SARS-CoV-2 antibodies was used to represent the cumulative infection rate of SARS-CoV-2 from the beginning of the epidemic to about 2 weeks before the investigation.

$$\text{Cumulative infection rate} = \frac{\text{Number of antibody positive individuals}}{\text{Number of participants}} \times 100\%$$

It should be noted that individuals whose antibody levels have not reached the positive level after infection cannot be detected.

Individual sampling weight calculation

In this study, the Probability-Proportional-to-Size (PPS) method was used for the sampling design, and the survey design weight was used to estimate the overall rate.

Base design weight,

$$W_{base} = \frac{\text{Number of residents in the district}}{\text{Total population in sampled communities} \times \text{number of communities}}$$

Demographic weight

Demographic weights were calculated by sample post-stratifying based on three demographic factors. The demographic weights of individuals depend on a specific age distribution in Wuhan (A_{age}) and participants (S_{age}), gender distribution in Wuhan (A_{sex}) and participants (S_{sex}), demographic distribution in 13 districts distribution in Wuhan (A_{dis}) and participants (S_{dis}). Participants were divided into 7 age groups as 0-14, 15-24, 25-34, 35-44, 45-54, 55-64, 65 and above", two gender groups and three district groups as "Hongshan, Hannan" group, "Huangpi, Qingshan, Caidian" group and "Others" group. To avoid over-adjusting, the sample size of every unit during the three-dimensional contingency table is greater than 49.

$$W_{adj} = \frac{A_{sexi} \times A_{agei} \times A_{disi}}{Population_i} \div \frac{S_{sexi} \times S_{agei} \times S_{disi}}{Sample_i}$$

To make the weighted sum of respondents in each stratum match the real population in Wuhan the total weight of the respondents (W_i) is $W_i = W_{adj} \times W_{base}$.

Questionnaire of the seroepidemiological study in Wuhan, China

Area: _____ District _____ Township (street)

Location of investigation: _____ community

Community number: □□

Current address: _____ Village (building) _____ Group (unit) No. _____.

Family number: □□ Personal number: □□

Survey object ID (automatically generated by the administrative system): _____

(township national standard code - community code - family code - personal code)

1. Name: _____ 2. Gender: □Male □Female

3. Mobile number: _____

4. Date of birth: _____/_____/_____ (DD/MM/YY)

5. ID number: _____

6. Occupation: □Scattered children □Nursery children □Student □Farmer □Workers □Administration officer □Healthcare worker □ Public health staff □Retirees □Commercial service personnel □Deliveryman □Takeout deliveryman □Security

   □Police □Taxi driver □Cleaning staff □Community workers □Other

7. Categories of community COVID-19 prevention and control personnel: (only for high risk population survey areas)

   □Community worker □Security □Police □Administration officer □Taxi driver

   □Volunteer □Cleaning staff □ Community healthcare worker □Other_____

8. Pregnant or not: □No □Yes, weeks of pregnancy: _____ Week _____Day

9. Did you give birth to a child in the past 6 months? □No □Yes, the delivery date: _____/_____/_____ (DD/MM/YY).

10. Underlying conditions (multi-choice): □None □Pulmonary diseases (such as asthma, COPD, pulmonary heart disease, pulmonary fibrosis, etc.) □Cancer chemotherapy □Hypertension □Diabetes □Cardiovascular and cerebrovascular diseases □Chronic kidney disease □Chronic liver disease □Immunodeficiency diseases □Unknow □Other_____.

11. Do you smoke? □Yes, I haven't quit smoking; □Yes, I've quit smoking; □No.

12. Have you had the following clinical symptoms since December 2019?

    Fever □No □Yes, the highest temperature_____℃, date of onset was _____/_____/_____ (DD/MM/YY).

    Cough □No □Yes, date of onset was _____/_____/_____ (DD/MM/YY).

    Anhelation □No □Yes, date of onset was _____/_____/_____ (DD/MM/YY).

    Rhinobyon □No □Yes, date of onset was _____/_____/_____ (DD/MM/YY).

    Rhinorrhea □No □Yes, date of onset was _____/_____/_____ (DD/MM/YY).

    Sore throat □No □Yes, date of onset was _____/_____/_____ (DD/MM/YY).

    Shortness of breath □No □Yes, date of onset was _____/_____/_____ (DD/MM/YY).

    Dyspnea □No □Yes, date of onset was _____/_____/_____ (DD/MM/YY).

    Myalgia □No □Yes, date of onset was _____/_____/_____ (DD/MM/YY).

Pneumonia □No □Yes (lung CT or X-ray changes), date of onset was _____/_____/_____ (DD/MM/YY).

Other symptoms □No □Yes, please specify_____, date of onset was _____/_____/_____ (DD/MM/YY).

13. Have you visited a medical institution because of fever or respiratory diseases since December 2019? □Yes □No.

13.1. If the answer is "Yes", the date was: □2019/12 □2020/1 □2020/2 □2020/3 □2020/4.

14. Have you been diagnosed with COVID-19 since December 2019? □Yes □No.

14. 1. If diagnosed, date of onset was _____/_____/_____ (DD/MM/YY).

(This information could be checked from the Hospital Information System).

15. Have you had the following travel or residence history since December 2019?

History of residence in Wuhan, □No □Yes, from_____/_____/_____ (DD/MM/YY) to _____/_____/_____ (DD/MM/YY).

History of travel to Wuhan, □No □Yes, from_____/_____/_____ (DD/MM/YY)

to_____/_____/_____ (DD/MM/YY).

History of residence in Hubei province outside Wuhan, □No □Yes, from_____/_____/_____ (DD/MM/YY) to_____/_____/_____ (DD/MM/YY).

History of travel to Hubei province outside Wuhan, □No □Yes, from_____/_____/_____ (DD/MM/YY) to _____/_____/_____ (DD/MM/YY).

History of living abroad, □No □Yes, name of the country _____,from _____/_____/_____ (DD/MM/YY) to _____/_____/_____ (DD/MM/YY).

History of travelling abroad, □No □Yes, name of the country_____, from _____/_____/_____ (DD/MM/YY) to _____/_____/_____ (DD/MM/YY).

16. Have you been in contact with anyone with fever or respiratory symptoms since December 2019?

□Yes □No.

16.1 If the answer is "Yes", date of the last contact was _____/_____/_____ (DD/MM/YY).

17. Have you ever been exposed to COVID-19 confirmed cases since December 2019?

□ Yes □ No

17.1 If the answer is "Yes", date of the last contact was _____/_____/_____ (DD/MM/YY). (This information could be checked from the local close contacts registration system)

18. Have you ever been exposed to asymptomatic SARS-CoV-2 infections since December 2019?

□ Yes □ No

18.1 If the answer is "Yes", the date of last contact was _____/_____/_____ (DD/MM/YY). (This information could be checked from the local close contacts registration system)

Name of the investigator: _____ Mobile number: _____

Date of the investigation: _____/_____/_____/ (DD/MM/YY)

19. Is the respondent a COVID-19 confirmed case after checking from the Chinese Notifiable Infectious Diseases Information System?

□ Yes, the clinical severity is: □ asymptomatic □ mild □ moderate □ severe □ critical

□ no

Name of the inspector: _____ Mobile number: _____

Date of the inspection: _____/_____/_____/ (DD/MM/YY)

Notes:
In addition to the personal information part, sampling information and test results should also be recorded into the "Serological Survey" information system. Please keep a good record of sampling information and test results.
1. The report information of antibody detection of blood samples mainly includes: antibody testing method; manufacturer, name, batch number, the expiration date of the reagent; qualitative and quantitative testing results, date of testing, person performed testing, person reviewed testing results, etc.
2. Among the areas in which respiratory tract swab specimen were collected, nucleic acid testing information should be recorded into the system, mainly including testing method, manufacturer, name, batch number, the expiration date of the reagent; manufacturer of nucleic acid extraction reagent, fluorescence quantitative test, PCR instrument, qualitative and quantitative testing results, date of testing, person performed testing, person reviewed testing results, etc.

Table S1. Positive rate of SARS-CoV-2 antibodies by sample collection as measured by pan-Igs, IgG, IgM, and IgA assays

Sample collection	N	Pan-Igs		IgG		IgM		IgA	
		N pos	Positive rate (%)	N pos	Positive rate (%)	N pos	Positive rate (%)	N pos	Positive rate (%)
Before 2019	102	0	0·00%	0	0·00%	0	0·00%	0	0·00%
Positive samples	56	56	100·00%	56	100·00%	48	85·71%	45	80·36%

N corresponds to the number of tested serum samples. N pos corresponds to the number of SARS-CoV-2 antibody-positive serum samples. The positive samples collected from COVID-19 patients were confirmed by using the western blot assay. SARS-CoV-2=severe acute respiratory syndrome coronavirus-2. Ig=Immunoglobin.

Table S2. The information of 56 serum samples with positive-antibody against SARS-CoV-2

Study population	qPCR	Patient ID	Sex	Age (years)	Date of symptom onset	Date of the sample collection	Pan-Igs (ECLIA)	ELISA (OD450)		
								IgM	IgA	IgG
Hospitalized patients from Lotus cohort in Wuhan Jinyintan Hospital	Positive	106	Female	63	2020/1/7	2020/1/18	92·28	0·8103	0·2520	0·8445
	Positive	107	Female	63	2020/1/7	2020/1/22	9·81	0·9129	1·0224	0·8269
	Positive	108	Female	63	2020/1/7	2020/1/27	1·04	0·9051	1·1974	0·8586
	Positive	109	Female	63	2020/1/7	2020/1/31	3·02	0·8111	0·9989	0·8730
	Positive	110	Male	26	2020/1/4	2020/1/18	49·62	0·7091	0·9324	0·8310
	Positive	115	Male	45	2020/1/7	2020/1/22	2·45	0·7846	0·2134	0·8806
	Positive	120	Male	72	2020/1/11	2020/1/27	19·05	0·3049	0·2063	0·3723
	Positive	121	Male	72	2020/1/11	2020/1/31	17·42	0·3015	0·2148	0·2809
	Positive	122	Female	63	2020/1/10	2020/1/18	18·58	0·3201	0·2014	0·3863
	Positive	123	Female	63	2020/1/10	2020/1/23	12·08	0·3270	0·2778	0·5444
	Positive	124	Female	63	2020/1/10	2020/1/27	9·71	0·3912	0·5704	0·8439
	Positive	125	Female	63	2020/1/10	2020/2/1	25·41	0·3734	0·3119	0·8518
	Positive	126	Female	68	2020/1/12	2020/1/18	1·92	0·3906	0·3820	0·6848
	Positive	127	Female	68	2020/1/12	2020/1/22	6·32	0·7707	0·2140	0·7860
	Positive	128	Female	68	2020/1/12	2020/1/27	23·10	0·6189	0·2148	0·7919
	Positive	129	Female	68	2020/1/12	2020/1/31	10·67	0·4659	0·8515	0·7881
	Positive	130	Female	56	2020/1/5	2020/1/18	1·13	0·3878	0·7335	0·7654
	Positive	135	Male	42	2020/1/5	2020/1/22	1·16	0·5702	0·7209	0·8122
	Positive	136	Male	42	2020/1/5	2020/1/27	39·99	0·5273	0·2104	0·8012
	Positive	137	Male	42	2020/1/5	2020/1/31	34·90	0·4866	0·2092	0·7724
	Positive	138	Female	48	2020/1/10	2020/1/18	21·63	0·5247	0·2098	0·5869
	Positive	140	Female	48	2020/1/10	2020/1/27	24·07	0·4588	0·2078	0·7511
	Positive	141	Female	48	2020/1/10	2020/1/31	4·98	0·7886	0·4325	0·7488
	Positive	142	Male	73	2020/1/6	2020/1/18	9·74	0·4448	0·2031	0·6882
	Positive	143	Male	73	2020/1/6	2020/1/22	11·61	0·6527	0·2086	0·7454
	Positive	146	Male	28	2020/1/10	2020/1/18	10·68	0·7886	0·2020	0·6810
	Positive	147	Male	28	2020/1/10	2020/1/22	69·19	0·7690	0·2163	0·6875
	Positive	148	Male	28	2020/1/10	2020/1/27	12·07	0·7103	0·1969	0·6792
	Positive	149	Male	28	2020/1/10	2020/1/31	8·00	0·7458	0·6860	0·6834
	Positive	151	Male	79	2020/1/7	2020/1/23	9·06	0·6960	0·3405	0·7016
	Positive	154	Male	53	2020/1/7	2020/1/27	18·42	0·8962	0·2151	0·7311
	Positive	155	Male	53	2020/1/7	2020/2/1	18·81	0·5927	0·2173	0·7415
	Positive	156	Male	38	2020/1/1	2020/1/18	101·90	0·7101	0·2122	0·6826
	Positive	159	Male	38	2020/1/1	2020/1/31	10·88	0·6359	0·2167	0·7219
	Positive	160	Male	38	2020/1/1	2020/2/2	22·30	0·7290	0·2149	0·6938
	Positive	161	Female	62	2019/12/27	2020/1/18	46·01	0·4197	0·3667	0·6783
	Positive	163	Female	62	2019/12/27	2020/1/27	25·08	0·3815	0·2945	0·6731
	Positive	164	Female	62	2019/12/27	2020/1/31	21·41	0·4044	0·2427	0·6761
	Positive	165	Male	42	2020/1/9	2020/1/18	27·22	0·3771	0·2179	0·2844
	Positive	167	Male	42	2020/1/9	2020/1/27	8·17	0·7881	0·2089	0·6957

	Positive	168	Male	42	2020/1/9	2020/1/31	3·78	0·9301	0·6608	0·6783
	Positive	169	Male	26	2020/1/7	2020/1/18	4·96	0·6237	0·4934	0·6789
	Positive	171	Male	26	2020/1/7	2020/1/27	15·60	0·4695	0·3079	0·6555
	Positive	172	Male	26	2020/1/7	2020/1/31	4·46	0·4320	0·2124	0·6907
	Positive	174	Male	58	2020/1/10	2020/1/22	2·10	0·8558	0·4673	0·688
	Positive	176	Male	52	2020/1/11	2020/1/22	53·58	0·4397	0·3885	0·7447
Recovered patients from Zhongnan Hospital of Wuhan University	Positive	12	Female	57	2020/1/31	2020/12/5	41·67	0·4665	0·1245	0·6288
	Positive	22	Female	79	2020/1/29	2020/12/5	54·43	0·2180	0·1745	0·5697
	Positive	23	Male	69	2020/1/15	2020/12/5	15·24	0·1480	0·1436	1·3637
	Positive	45	Female	51	2020/1/28	2020/12/5	145·50	0·2412	0·1496	0·9029
	Positive	47	Male	42	2020/1/15	2020/12/5	69·48	0·1846	0·189	0·5991
	Positive	48	Female	33	2020/2/1	2020/12/5	44·49	0·2587	0·1194	0·7903
	Positive	49	Female	69	2020/2/4	2020/12/5	102·30	0·2455	0·1850	1·0801
	Positive	51	Male	31	2020/1/8	2020/12/5	34·44	0·2485	0·1878	1·4107
	Positive	52	Female	31	2020/1/27	2020/12/5	27·12	0·5950	0·0909	0·3472
	Positive	54	Female	27	2020/1/21	2020/12/5	47·58	0·2807	0·1328	0·3592

qPCR=Fluorescence quantitative PCR; ECLIA= Electrochemiluminescence immunoassay; ELISA= Enzyme-linked immunosorbent assay; Ig= Immunoglobulin; Lotus = Lopinavir Trial for Suppression of SARS-Cov-2 in China

Table S3. Goodness of fit test of the population by age, gender, and district

Variables	Population	Participants	Proportion by population (%)	Proportion by participants (%)	Goodness of fit values
Age*					
0–14	1 256 552	1 259	0·11%	13·19%	0·26
15–24	1 361 497	714	12·29%	7·48%	3·08
25–34	2 330 422	1 521	21·03%	15·94%	1·63
35–44	1 518 966	1 881	13·71%	19·71%	1·83
45–54	1 937 933	1 691	17·49%	17·72%	0·00
55–64	1 436 455	1 502	12·96%	15·74%	0·49
≥65	1 239 171	974	11·18%	10·21%	0·09
Gender†					
Male	5 912 919	4 658	53·36%	48·82%	0·39
Female	5 168 077	4 884	46·64%	51·18%	0·44
District‡					
Jiang'an	962 695	931	8·69%	9·76%	0·12
Jianghan	729 704	650	6·59%	6·81%	0·01
Qiaokou	868 702	767	7·84%	8·04%	0·01
Hanyang	664 202	633	5·99%	6·63%	0·06
Wuchang	1 282 800	1 028	11·58%	10·77%	0·06
Qingshan	528 894	564	4·77%	5·91%	0·22
Hongshan	1 677 298	842	15·14%	8·82%	4·52
Dongxihu	584 803	493	5·28%	5·17%	0·00
Hannan	427 916	627	3·86%	6·57%	1·12
Caidian	469 490	655	4·24%	6·86%	1·01
Jiangxia	962 000	197	8·68%	2·07%	21·21
Huangpi	1 011 897	1 047	9·13%	10·97%	0·31
Xinzhou	910 595	1 108	8·22%	11·61%	0·99

* χ^2=7·126, p > 0·05; † χ^2 = 0·83, p > 0·05; ‡ χ^2 = 29·620, p < 0·01

Table S4. The number of SARS-CoV-2 antibody-positive family by family size

Family size (person)	Number of family (%)	Number of positive family	Positive rate (95% CI)
1	816 (22·95%)	64	7·84% (6·00–9·68)
2–3	1 984 (55·79%)	209	10·53% (9·18–11·89)
≥4	756 (21·26%)	118	15·61% (13·02–18·20)

CI=confidence interval

Table S5. The seroprevalence and population density in districts in Wuhan city, China

Districts	Number of Population	Population density (Number of persons/km^2)	Seroprevalence (95% CI)
Qiaokou	868 702	20 952·77	13·08% (10·69–15·47)
Jiang'an	962 695	13 703·84	11·12% (9·10–13·14)
Hanyang	664 202	5954·83	7·88% (5·78–9·98)
Jianghan	729 704	25 793·71	7·83% (5·76–9·90)
Qingshan	528 894	6572·56	5·54% (3·65–7·43)
Hongshan	1 677 298	7606·79	5·62% (4·06–7·18)
Wuchang	1 282 800	11 904·23	5·15% (3·80–6·50)
Jiangxia	962 000	476·64	5·07% (2·01–8·13)
Dongxihu	584 803	1170·28	3·15% (1·61–4·69)
Xinzhou	910 595	606·80	2·35% (1·46–3·24)
Huangpi	1 011 897	448·40	1·62% (0·86–2·38)
Caidian	469 490	429·32	1·49% (0·56–2·42)
Hannan	427 916	1485·82	0·73% (0·06–1·40)

CI=Confidence interval

Table S6. Antibody positive rates for IgM, IgA, IgG, and NAbs against SARS-CoV-2 by sex and age

	IgM N (%, 95% CI)	IgA N (%, 95% CI)	IgG N (%, 95% CI)	NAbs N (%, 95% CI)
Sex				
Male (n=4658)	25 (0·54%, [0·33–0·75])	40 (0·86%, [0·59–1·12])	217 (4·66%, [4·05–5·26])	90 (1·93%, [1·54–2·33])
Female (n=4884)	44 (0·90%, [0·64–1·17])	44 (0·90%, [0·64–1·17])	315 (6·45%, [5·76–7·14])	122 (2·50%, [2·06–2·94])
Age (years)				
0–5 (n=303)	4 (1·32%, [0·03–2·61])	2 (0·66%, [0·00–1·57])	14 (4·62%, [2·26–6·98])	7 (2·31%, [0·62–4·00])
6–11 (n=682)	2 (0·29%, [0·00–0·70])	2 (0·29%, [0·00–0·70])	23 (3·37%, [2·02–4·73])	13 (1·91%, [0·88–2·93])
12–17 (n=485)	4 (0·82%, [0·02–1·63])	0 (0·00%, [0·00–0·00])	16 (3·30%, [1·71–4·89])	9 (1·86%, [0·65–3·06])
18–44 (n=3905)	30 (0·77%, [0·49–1·04])	22 (0·56%, [0·33–0·80])	214 (5·48%, [4·77–6·19])	69 (1·77%, [1·35–2·18])
45–65 (n=3340)	22 (0·66%, [0·38–0·93])	41 (1·23%, [0·85–1·60])	202 (6·05%, [5·24–6·86])	90 (2·69%, [2·15–3·24])
≥66 (n=827)	7 (0·85%, [0·22–1·47])	17 (2·06%, [1·09–3·02])	63 (7·62%, [5·81–9·43])	24 (2·90%, [1·76–4·05])
Total (n=9542)	69 (0·72%, [0·55–0·89])	84 (0·88%, [0·69–1·07])	532 (5·58%, [5·11–6·04])	212 (2·22%, [1·93–2·52])

CI= confidence interval. Ig=Immunoglobulin. NAbs=Neutralizing antibodies.
SARS-CoV-2=severe acute respiratory syndrome coronavirus-2.

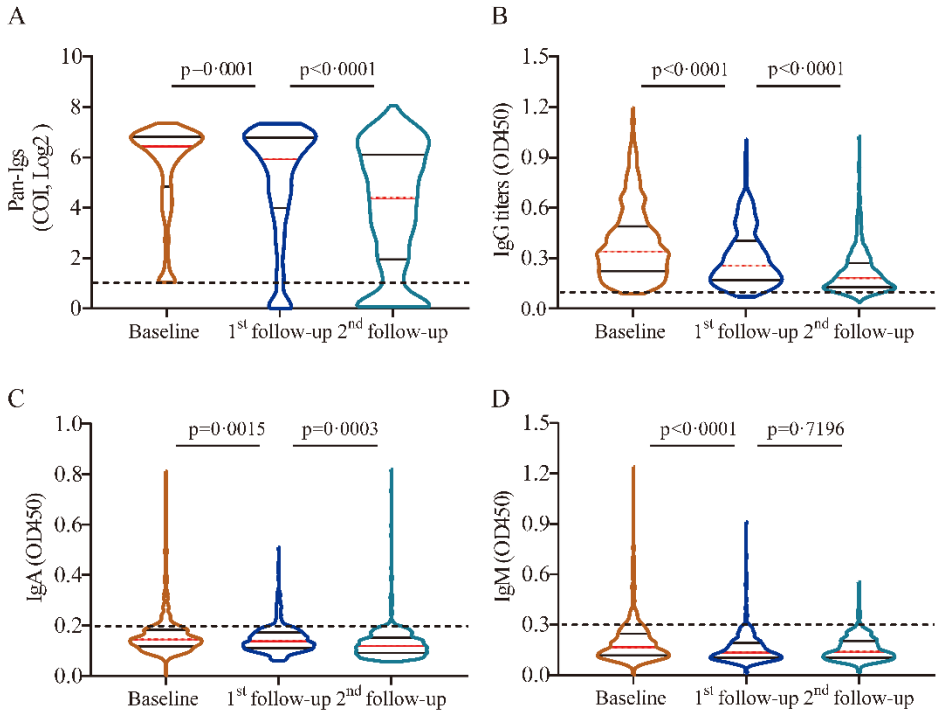

Figure S1. Temporal changes of antibody titers against SARS-CoV-2 at baseline, the 1st follow-up, and the 2nd follow-up

Temporal changes of antibody titers of pan-Igs (A), IgG (B), IgA (C), and IgM (D) are shown. The dashed lines indicate the thresholds for a test to be declared positive. Red lines denote the median of antibody titers. Black lines denote the interquartile range. The number of participants at baseline, the 1st follow-up, and the 2nd follow-up was 532, 363, and 454, respectively. Ig= immunoglobin. OD=optical density.

Table S7. Relative change in serum antibody levels between baseline and second follow-up for IgM, IgA, and IgG against SARS-CoV-2

	Baseline	First follow-up	Second follow-up	Relative change (%)
IgM	69 (13·0%)	14 (3·9%)	84 (15·8%)	88.5%
IgA	84 (15·8%)	36 (9·9%)	16 (3·5%)	77.8%
IgG	532 (100%)	354 (97·5%)	413 (91·0%)	9.0%

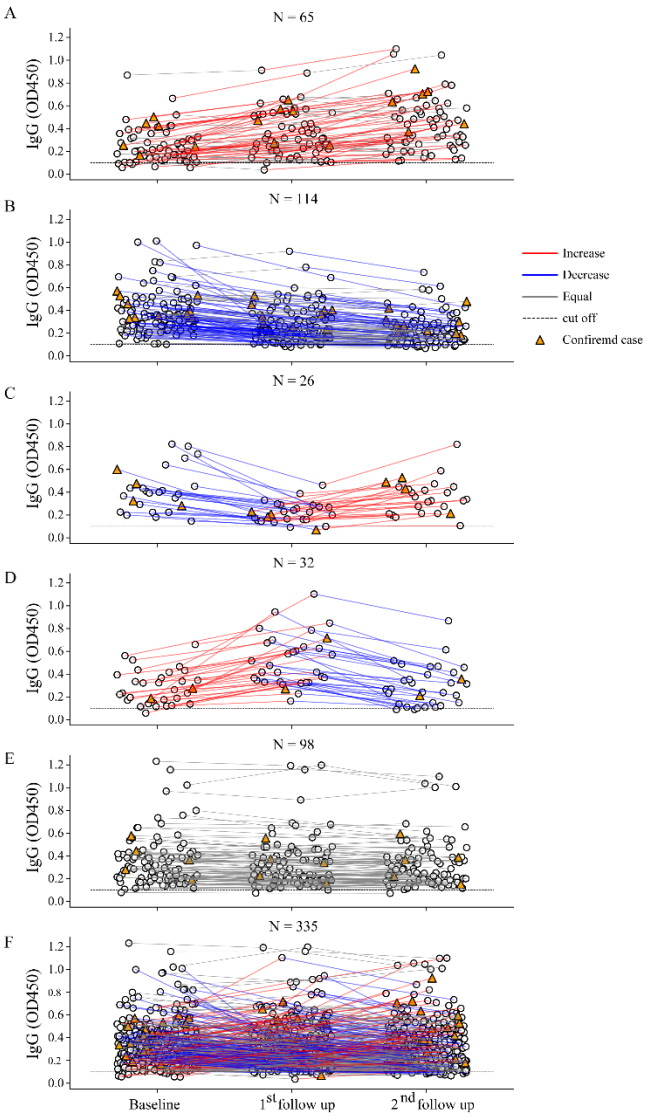

Figure S2. Dynamic changes of IgG levels at baseline, 1st follow-up, and 2nd follow-up at the individual level.

Kinetics of IgG antibody among the 335 individuals who had three consecutive plasma samples. The IgG antibody titers increased continuously in 65 individuals (A), decreased in 114 individuals (B), initially decreased, then increased in 26 individuals (C), initially increased and then decreased in 32 individuals, kept stable in 98 individuals during the follow-ups (E), and the overall changes in all 335 participants (F).

Table S8. The linking of sustained and non-sustained neutralizing antibodies by sex, age, and symptoms

	Sustained*	Non-sustained†	*p*-value
Sex			
Male	46	83	0·36
Female	85	121	
Age (years)			
≤65	111	178	0·83
>65	19	27	
Symptomatic			
Yes	46	36	<0·0001
No	84	169	

*Sustained represents the presence of antibodies across the baseline and follow-ups.

†Non-sustained represents negative antibody detections during follow-ups